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Supplemental information

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Supporting Information

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Running title: cAMP permeation through hemichannel

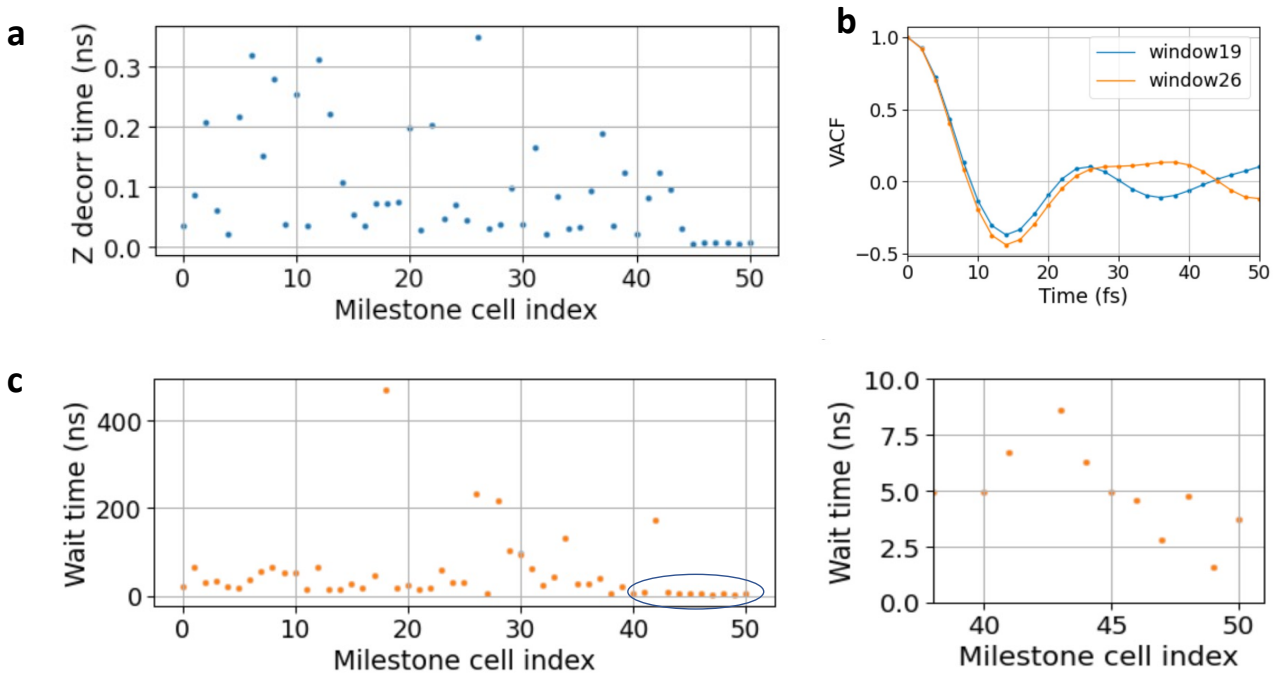
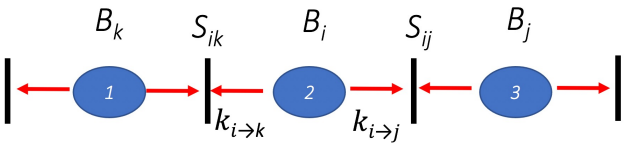
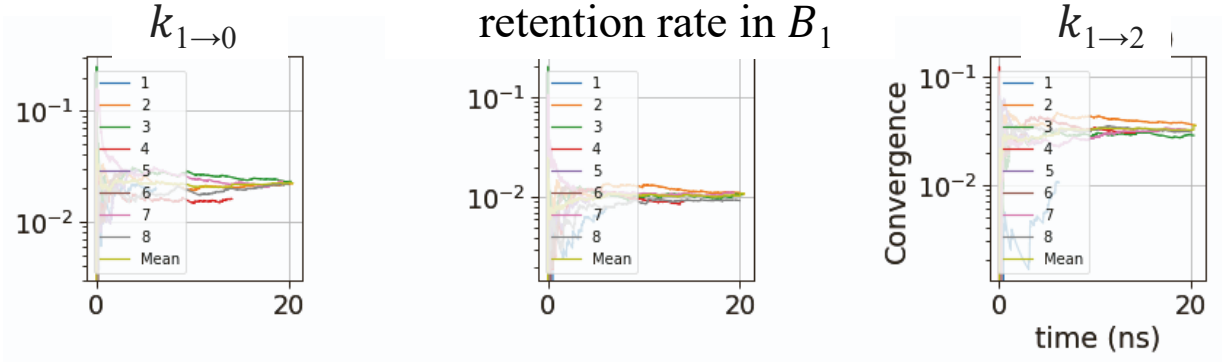


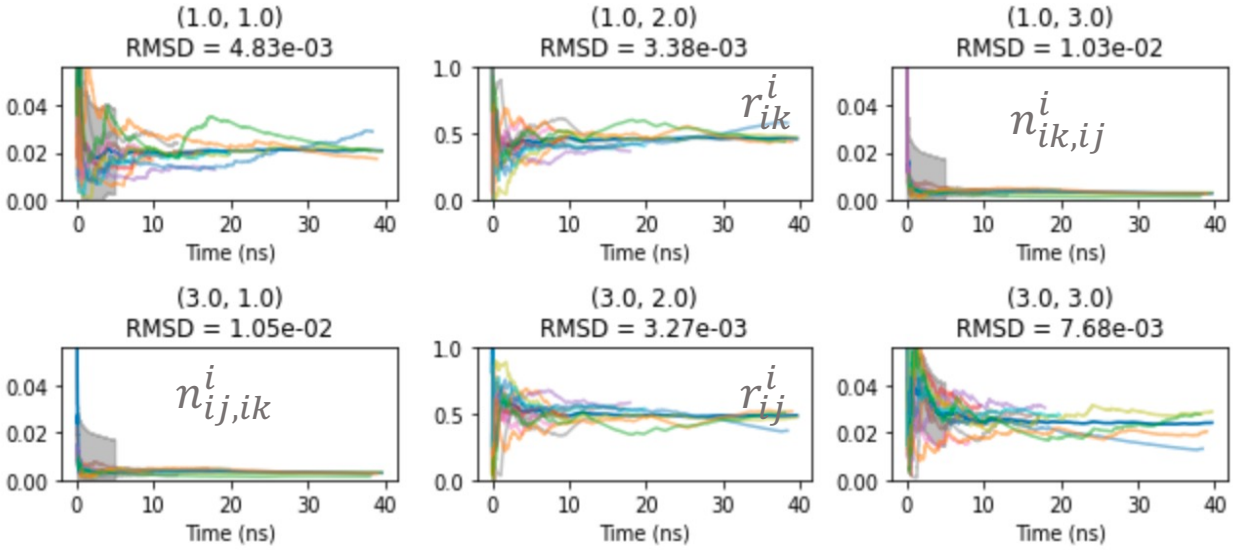
Figure S1. a) cAMP z-position decorrelation time in each milestone cell. **b**) cAMP z-velocity autocorrelation functions of window 19 (slowest position decorrelation) and window 26 (longest wait time). **c**) Average wait time of transition event in each milestone cell. The circled region is zoomed in on the right.


B_k
S_{ik}
B_i
S_{ij}
B_j
1
2
3
$k_{i\to k}$
$k_{i\to j}$

a


$k_{1\to 0}$
retention rate in B_1
$k_{1\to 2}$
Convergence
time (ns)
Mean

b


(1.0, 1.0)
RMSD = 4.83e-03
(1.0, 2.0)
RMSD = 3.38e-03
r^i_{ik}
(1.0, 3.0)
RMSD = 1.03e-02
$n^i_{ik,ij}$
(3.0, 1.0)
RMSD = 1.05e-02
$n^i_{ij,ik}$
(3.0, 2.0)
RMSD = 3.27e-03
r^i_{ij}
(3.0, 3.0)
RMSD = 7.68e-03
Time (ns)

c

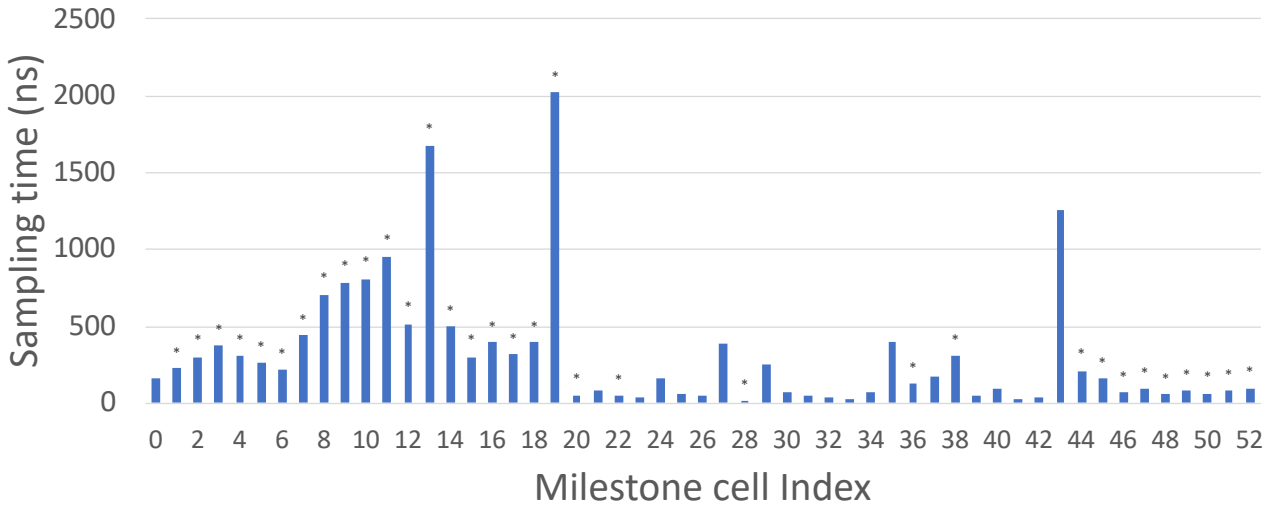

Sampling time (ns)
Milestone cell Index

Figure S2. a) PMF convergence plots of a Voronoi cell for index 01 (B_1) over time. The left and right plots represent the probability of the attempted escape to the left or right milestone states, $k_{1\to0}$ and $k_{1\to2}$. The middle plot is the retention rate inside the cell. Colors represent different replicas and the mean of all the replicas. **b**) convergence plots for MFPT for milestoning cell index 2 (B_2). The upper right and lower left panels correspond to the frequencies of transitions from cell 1 to cell 3, and from cell 3 to cell 1, respectively ($n^i_{ij,ik}$ and $n^i_{ik,ij}$). The upper and lower center panels correspond to the percentage of time spent in cell 2 after last touching cell 1 and 3 (r^i_{ij}), respectively. The other two entries are not used in analysis directly but would correspond to re-entering cell 1 or 3 after last visiting that same cell previously. The final 10 ns (last 10,000 frames) windowed relative RMSD is also given at the top of each panel as a measure of the degree of convergence for the corresponding rate matrix entry components. The convergence plots of all 53 cells are available on Github repository https://github.com/LynaLuo-Lab/Connexin-cAMP-milestoning. **c**) Total sampling time (ns) for each milestone cell (star indicating multi-replicas).

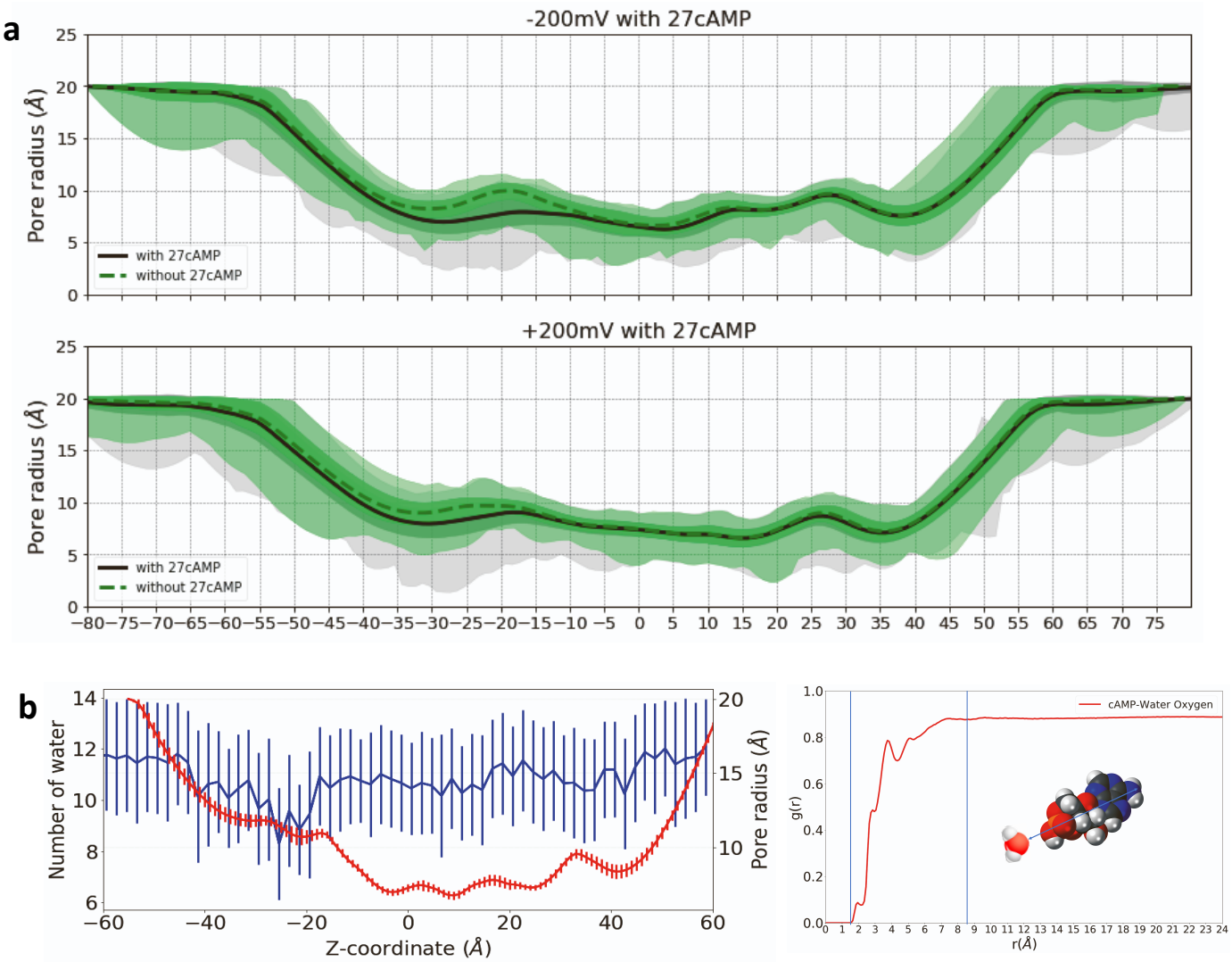


Figure S3. a) Pore radius profiles from two simulations under +/-200 mV, calculated using Hole program. The black line is the average value over last 1 μs, with 1.2 ns interval between snapshots. The dark grey shade represents the +/- standard deviation, the light grey shade represents the minimum and maximum radius values. The dashed line and green shade are the pore radius profile with protein only. **b**) Average and standard deviation of the number of water molecules within 2.15 Å of cAMP (blue) at each z-position of the channel lumen computed from milestoning simulations, aligned with the average and standard deviation of pore radius (red). The 2.15 cutoff distance is determined from the radial distribution function of cAMP and water oxygen computed from trajectory of cAMP in bulk (right).

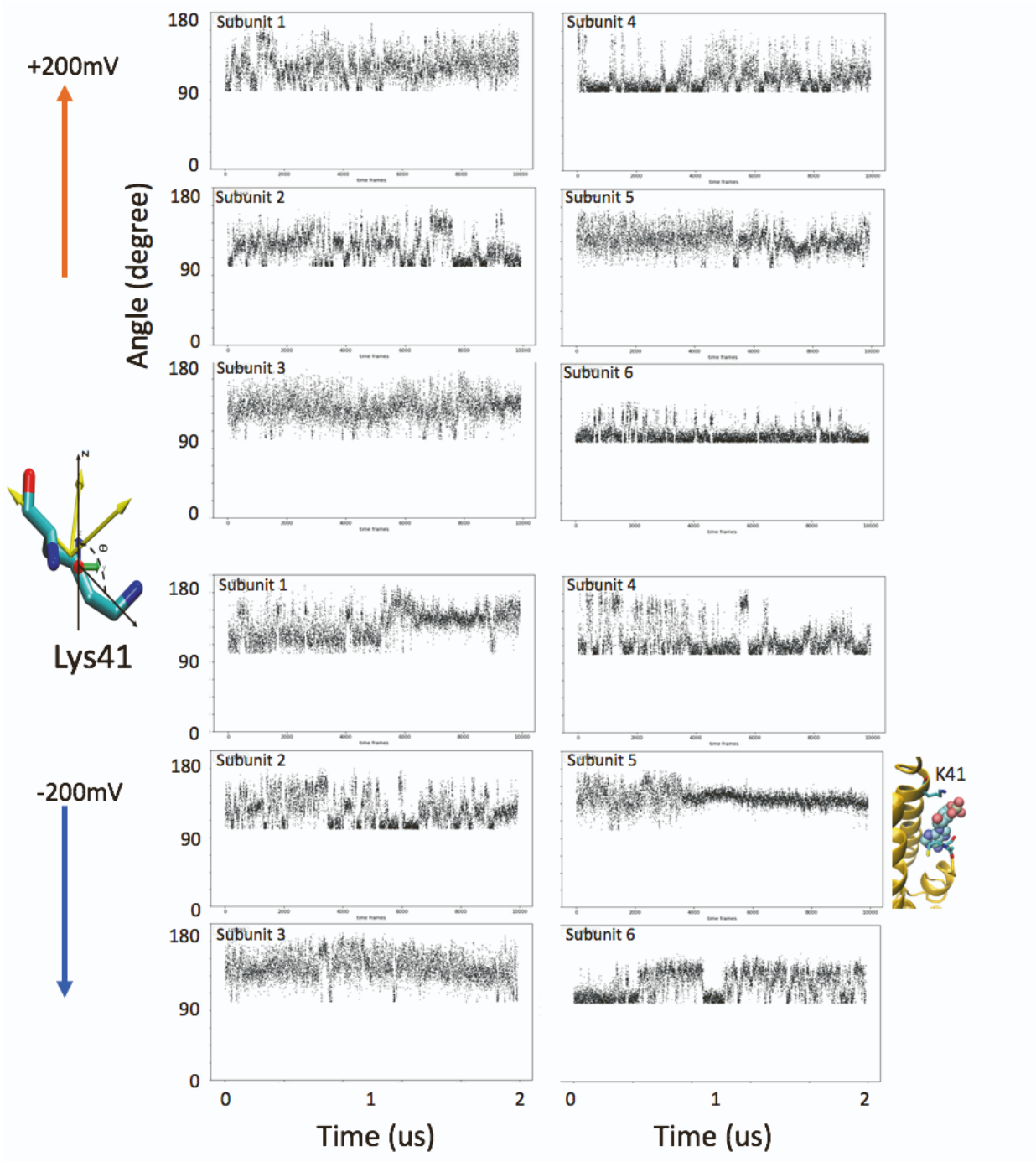


Figure S4a. Fluctuation of the angles between the principal axis of Lys41 and z-vector in each subunit under +/-200 mV. Representative Lys residue shown in the licorice model with its principal axes and the angle plotted.

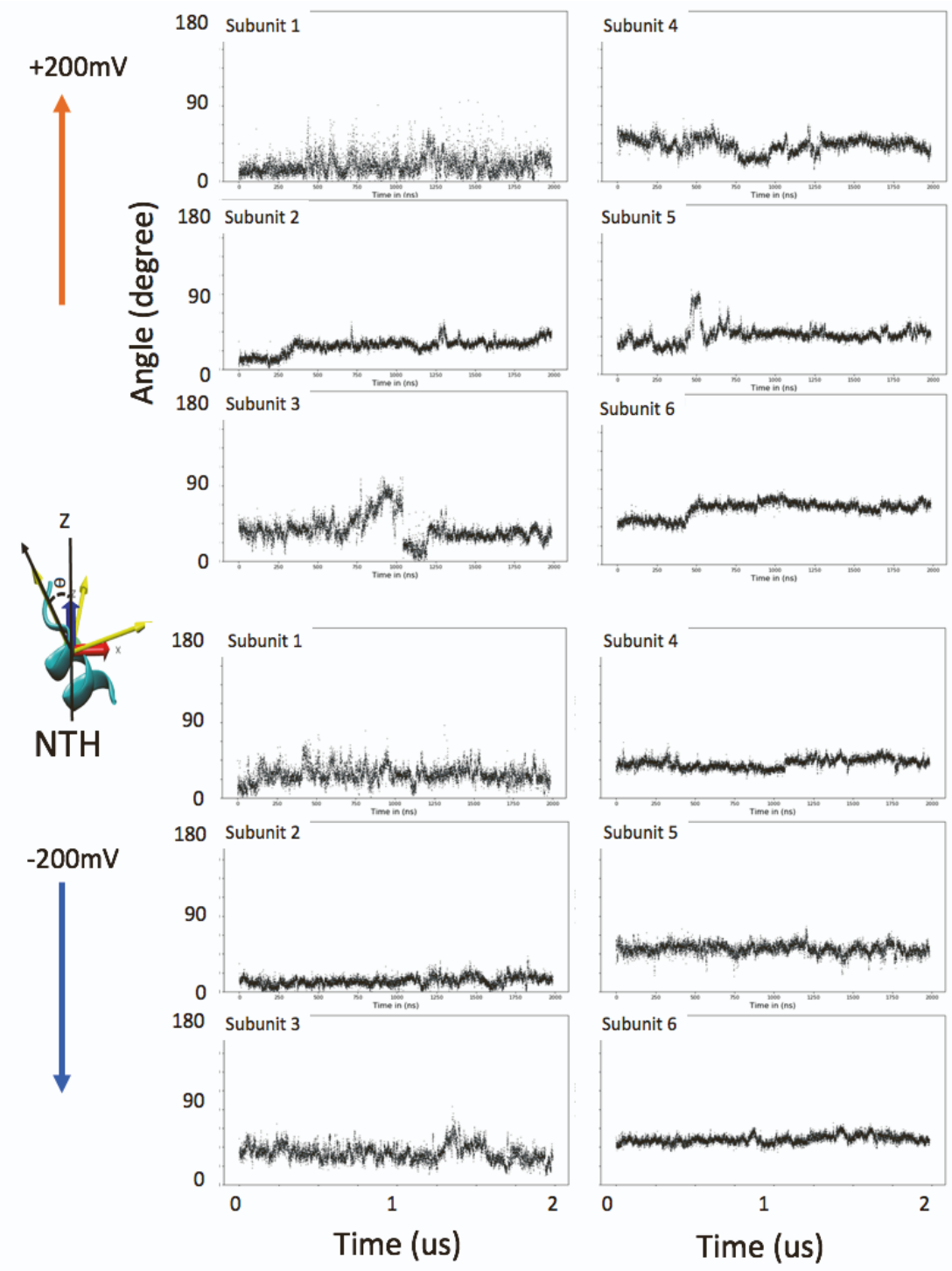


Figure S4b. Fluctuation of the angles between the principal axis of NTH (1-11) and z-vector in each subunit under +/-200 mV. Representative NTH shown in the new cartoon model with its principal axes and the angle plotted.

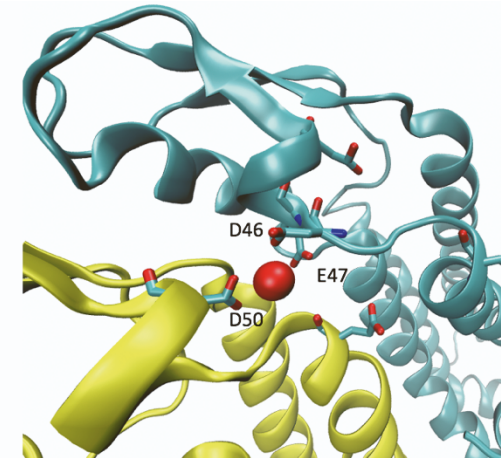


Figure S5. One Mg^{2+} (red ball) binds to the calcium-binding site (D50, D46, E47) for 1.5 μs during the voltage simulation, which contains 27 Mg^{2+} in the whole system.

Table S1. Optimized CHARMM general force field (CGenFF) parameters for cAMP, available at https://github.com/LynaLuo-Lab/Connexin-cAMP-milestoning.

BONDS						
CG3RC1	OG3R60	334.3	1.411			
OG3R60	PG1	237	1.61			
ANGLES						
CG3RC1	CG321	OG3R60	75.7	110.1		
CG321	CG3RC1	OG3C51	45	111.5		
CG3C51	CG3RC1	OG3R60	58	106.5	8	2.561
CG3RC1	CG3RC1	OG3R60	53.35	111	8	2.561
OG3R60	CG3RC1	HGA1	45.9	108.5		
CG3C51	OG3C51	CG3RC1	170	109		
CG321	OG3R60	PG1	20	120	35	2.33
CG3RC1	OG3R60	PG1	20	120	35	2.33
OG2P1	PG1	OG3R60	98.9	107.5		
OG3R60	PG1	OG3R60	80	104.3		
DIHEDRALS						
OG3R60	CG321	CG3RC1	CG3RC1	0.6	1	0
OG3R60	CG321	CG3RC1	CG3RC1	0.45	2	0
OG3R60	CG321	CG3RC1	CG3RC1	0.7	3	0
OG3R60	CG321	CG3RC1	OG3C51	3.4	1	180
OG3R60	CG321	CG3RC1	HGA1	0.195	3	0

DIHEDRALS						
OG311	CG3C51	CG3RC1	OG3R60	0	3	0
HGA1	CG3C51	CG3RC1	OG3R60	0.195	3	0
CG3C51	CG3C51	OG3C51	CG3RC1	0.5	3	0
NG2R51	CG3C51	OG3C51	CG3RC1	0	3	0
HGA1	CG3C51	OG3C51	CG3RC1	0.3	3	0
CG321	CG3RC1	CG3RC1	OG3R60	0.15	3	0
OG3C51	CG3RC1	CG3RC1	OG3R60	1.2	3	0
OG3R60	CG3RC1	CG3RC1	HGA1	0.15	3	0
CG321	CG3RC1	OG3C51	CG3C51	0.3	3	0
CG3RC1	CG3RC1	OG3C51	CG3C51	0	3	0
HGA1	CG3RC1	OG3C51	CG3C51	0.3	3	0
CG3C51	CG3RC1	OG3R60	PG1	2.553	1	180
CG3RC1	CG3RC1	OG3R60	PG1	1.5	1	180
CG3RC1	CG3RC1	OG3R60	PG1	0.625	2	180
CG3RC1	CG3RC1	OG3R60	PG1	0.488	1	180
CG3RC1	CG3RC1	OG3R60	PG1	0.972	3	180
HGA1	CG3RC1	OG3R60	PG1	1.903	1	0
HGA1	CG3RC1	OG3R60	PG1	0.089	3	180
CG321	OG3R60	PG1	OG2P1	0.1	3	0
CG321	OG3R60	PG1	OG3R60	2.248	1	180
CG321	OG3R60	PG1	OG3R60	0.261	2	0
CG321	OG3R60	PG1	OG3R60	2.64	3	180

HGA2	CG321	CG3RC1	OG3C51	0.16	3	0
CG3RC1	CG321	OG3R60	PG1	0.002	1	180
CG3RC1	CG321	OG3R60	PG1	1.14	2	180
CG3RC1	CG321	OG3R60	PG1	0.086	3	0
HGA2	CG321	OG3R60	PG1	0.599	3	0
CG3RC1	CG3C51	CG3C51	NG2R51	0	3	0
CG3RC1	CG3C51	CG3C51	OG3C51	0	3	0
CG3C51	CG3C51	CG3RC1	OG3R60	2	3	180
CG3C51	CG3C51	CG3RC1	OG3R60	0.4	5	0
CG3C51	CG3C51	CG3RC1	OG3R60	0.8	6	0

Table S2. Description of the computational systems

	Voltage	term patching	Disulfide bonds	Box (A^3)	Number (concentration*)			
					atoms	cAMP	K+/Mg2+/Cl-	POPC/water
Anton2	+/- 200mV	ACE CT1	C53-C180 C60-C174 C64-C169	122× 122x 154	237030	27 (26.5 mM)	82/27/163 (80/26/160 mM)	323/56569
Milestoning	0 mV	ACE CT1	C53-C180 C60-174 C64-C169	121x 121x 155	234652	1 (1 mM)	82/1/137 (81/1/135 mM)	324/56191

*Concentrations were calculated based on number of water molecules.

Table S3. Transition time and dwell time (ns) from 2 μs trajectories at +200 mV and -200 mV.

Transition Event	+200mV (inward flux)			-200mV (outward flux)		
	Transition Time -50<z<50	Dwell time -50<z<-20	Barrier crossing -20<z<50	Transition Time -50<z<50	Dwell time -50<z<-20	Barrier crossing -20<z<50
1	222	24	198	160	110	50
2	1218	1058	160	242	210	32
3	416	308	108	290	50	240
4	282	248	34	1496	1400	96
5	542	498	44	230	162	68
6	290	149	140	630	570	60
7	192	120	72	384	350	34
8	454	222	232	152	100	52

9	426	150	276	1410	1228	182
10				198	76	122
11				390	150	240
12				445	350	95
Raw mean	449	308	140	502	396	105
Sample mean*	448	305	143	510	398	106
95% CI*	204-1646	149-940	79-330	249-1571	205-1086	54-290

*The sample mean and confidence intervals are based on Maximum Likelihood Estimate by fitting the exponential distributions (see code at https://github.com/LynaLuo-Lab/MD-data-uncertainty-analysis/blob/master/confidence_interval_exponential.ipynb).